REMARKS

I. Status of the Claims

Claims 1-42 are pending in the instant case. The claims were restricted to two inventions as discussed in further detail herein below. Claims 1-38, the claims of Group I, were elected with traverse for prosecution in the instant application and these claims were rejected under 35 U.S.C. §103(a). Applicants respectfully traverse the rejections for the reasons presented below.

II. Response to Restriction Requirement

The Examiner required that the present invention be restricted, under 35 U.S.C. §121, to one of the following inventions:

Group I: Claims 1-38 drawn to methods of determining cytotoxicity

Group II: Claims 39-42 drawn to kits for determining cytotoxicity.

During a telephone conversation with the Examiner on 1/18/01, the undersigned representative provisionally elected the claims of Group I, *i.e.*, claims 1-38. This election was made ***with traverse.***

Generally speaking, the subject matter of the claims of Group I is drawn to methods of determining cytotoxicity and the subject matter of the claims of Group II is directed to kits for accomplishing such methods. As such, Applicants believe that a search designed to identify art relating to a method that determines a first, second and third indicator of cell health, each in the presence of four or more concentrations of the chemical compound (*i.e.*, the methods of Group I) will identify art related to kits that may be used in such an endeavor. Likewise, the patentability issues that arise during prosecution should be similar.

Given the above discussion, Applicants respectfully solicit the Examiner's discretion in rejoining the claims of Group II, with those of Group I, because searching and examining these claims together would not be unduly burdensome. See MPEP

§803 ("If the search and examination of an entire application can be made without serious burden, the examiner must examine it on the merits, even though it includes claims to distinct or independent inventions."). As such, Applicants respectfully request that the restriction requirement, in respect to Groups I and II, be withdrawn and these groups be examined simultaneously.

II. The rejection of claims 1-38 under 35 U.S.C. § 103(a), for being unpatentable over Kangas *et al.* (Med Biol, 62:338-343, 1984) in view of Redick *et al.* (J Biol Chem, 257:15200-3) and Connors *et al.* (Biochem Pharmacol) 24:2217-24, 1975) should be withdrawn.

The Examiner rejected claims 1-38 under 35 U.S.C. §103(a), for being obvious over Kangas *et al.* (Med Biol., 62:338-343, 1984) in view of Redick *et al.* (J Biol Chem, 257:15200-3) and Connors *et al.* (Biochem Pharmacol) 24:2217-24, 1975). According to the Official Action, Kangas and Redick teach ATP assays, MTT assays, Alamar Blue assays and Rhodamine assays. The Examiner goes on to state that Kangas teach a method of predicting the cytotoxicity of a chemical compound by measuring a number of different assays and that Kangas teaches the treatment of cancer. The Examiner states that Redick teaches choosing liver cell lines and Connors teaches *in vivo* methods of screening for anti-cancer agents and predicting the cytotoxicity of chemical compounds. The Examiner asserts that "one of skill in the art would have been motivated to modify the teaching of Kangas by the addition of liver cell lines as taught by Redick and *in vivo* as taught by Connors" to facilitate the use of the techniques in real world pharmaceutical compositions instead of merely *in vitro* testing. Applicants traverse the rejection at least for the reasons provided below and request reconsideration.

In order for a case of *prima facie* obviousness to be properly established, it must be shown that the art contains a teaching of each of the elements of the claimed invention. Moreover, in addition to a requirement for a teaching all the claim features, a given combination of references must provide some suggestion or motivation to modify the reference(s) and there must be some reasonable expectation of the success of such modification of the reference. *In re Vaeck*, 20 USPQ2d 1438,

1445 (Fed. Cir. 1991). The motivation and the reasonable expectation of success must come from the art and not from the Applicants' own disclosure. As stated in MPEP §2143, all three of the above criteria ***must*** be met in order to properly establish *prima facie* obviousness. It is the Applicants' position that none of these criteria are met by the combination of Kangas *et al.* in view of Redick *et al.* and Connors *et al.*

The present invention is an *in vitro* method of predicting the *in vivo* cytotoxicity of a chemical compound and involves performing multiple assays to develop a cytotoxicity profile for the chemical compound to accurately assess the cytotoxicity of the compound *in vivo (e.g.,* see page 23 of the specification). In particular, the invention involves conducting at least three assays with different biochemical endpoints in order to predict the *in vivo* toxicity of the compound. None of the references cited either alone, or in combination, provide such a teaching.

The teachings of Kangas are entirely directed to evaluating a method of using ATP to measure the viability of a cell. Kangas describes the validation of an ATP bioluminescence assay by contrasting the results of the ATP assay individually with the assays for cell number, viability, thymidine incorporation and stem cell assay. However, at no point does Kangas teach or suggest that it would be desirable or possible to better predict the *in vivo* cytotoxicity of an agent by performing at least three of these assays, determining a C_{tox} concentrations from a combination of these assays and generating a cytotoxicity profile of a cytotoxic agent as is taught by the present invention. The entire focus of Kangas is to produce one ". . .universal method-applicable to any cell line. . .to enable the evaluation of cell growth as well as cell death." (See page 338 first full paragraph under INTRODUCTION). Thus, rather than teaching the use of ***multiple assays*** to produce a cytotoxicity profile of a cytotoxic agent (as the present invention contemplates), Kangas is aiming to produce one uniform assay in which ***only one*** parameter *i.e.* bioluminescence of cellular ATP, is measured. In effect, Kangas is teaching away from the present invention and proposes that ". . .the ATP-bioluminescence method ***is a powerful alternative*** to any other cell growth estimation method in vitro. . ." (***emphasis added***; Kangas Abstract, page 338).

Neither the Redick nor the Connors reference does anything to rehabilitate the flaws of Kangas. Redick does not teach an *in vitro* GST leakage monitoring assay in which the leakage of GST into the media of a cell culture is determined, but rather reports the immunohistochemical localization of three isozymes of GST, transferases B, C, and E, in the liver of the rat using sheep antibodies raised against these three isozymes of hepatic GST. Hence, this reference provides background for determining the presence of GST on a histochemical slide and suggests that this enzyme may be used a marker for liver damage, in tissue sections. It provides no teaching or direction of monitoring GST leakage into cell culture media. As such, the combination of Kangas and Redick still do not provide the requisite elements of claim 1 in that it does not provide a teaching of conducting at least three assays to determine the C_{tox} of an agent.

The Connors *et al.* reference points out various limitations of applying *in vitro* assays to test for and predict the *in vivo* cytotoxicity of agents. It seems to be Connors' position that *in vitro* tests are of limited use in such an endeavor. In these discussions of the limited usefulness of *in vitro* assays, Connors focuses on determining the cytotoxic effects of an agent in single assays, and not using the composite data generated through the use of multiple assays assessing the effects of an agent on a plurality of parameters as is required by the claims of the present invention. Connors proposes that cytotoxicity should be tested *in vivo* using animal models. The present invention overcomes ineffectiveness of single assays in predicting the cytotoxicity discussed in Connors by using the *in vitro* cytotoxicity screens of the present invention. Moreover, the cytotoxicity screens of the present invention alleviates the problems of expense and difficulty of obtaining animal models (see specification page 1-2 for discussion of the drawbacks of using animal models for initial cytotoxicity screening.) The combination of Kangas, Redick and Connors fails to teach or suggest that it is either desirable or even possible to predict *in vivo* cytotoxicity using a cluster of at least three *in vitro* assays. Hence, the cited references do not provide a teaching of all the elements of the claimed methods.

Moreover, the Connors reference, on its face, disparages the usefulness of *in vitro* assays for such predictions and suggests instead that one of skill in the art

should employ *in vivo* assays. In addition, the Kangas reference specifically teaches that the cytotoxicity of agents should be assessed using only one uniform assay in which ***only one*** parameter *i.e.* bioluminescence of cellular ATP, is measured. Thus, not only does the combination of all the references not teach all the claimed elements, two out of the three references cited actually teach away from the present invention. As such, viewing these references in combination, one of skill in the art would not be motivated to conduct multiple *in vitro* assays in order to predict *in vivo* cytotoxicity of an agent.

Furthermore, even if one of skill in the art were to combine all the references and fortuitously conduct a method of the present invention using at least three *in vitro* assays simultaneously to predict the *in vivo* cytotoxicity of a given agent, that individual would still not have a reasonable expectation of success of achieving such a result because at least two of the references actually teach away from such a result. The Examiner has failed to cite any basis in the prior art for suggesting that the Applicants' invention could achieve the excellent results that it does in predicting the *in vivo* cytotoxicity of an agent using a cluster of *in vitro* assays.

In summary, Applicants submit that the cited references, either alone or in combination, fail to teach or suggest all the elements of Claim 1; there is no motivation or suggestion to combine the teachings of the cited references and even if one of skill in the art were to combine the references, there would be no reasonable expectation of success of achieving the claimed invention. The references do not establish the alleged *prima facie* obviousness of the invention of Claim 1.

Claims 2-38 ultimately all depend from Claim 1 and thus are also patentable over the cited references. The dependent claims are directed to numerous additional aspects and further define the invention claimed in claim 1. The Examiner has not cited and Applicants see no evidence of a teaching of identifying the NOEL concentrations which is the highest concentration of the chemical compound at which a measurable toxic effect of the chemical is not observable (required element of claim 3); determining a TC50 for each of the indicators of cell health (required element of claim 4); a method of identifying a lead compound for drug development (claim 31) or numerous other features of the other dependent claims. Such teachings must come

from the prior art and must not be generated through a hindsight construction following the teachings of the present invention because using the Applicants own disclosure as a roadmap to identify individual and disparate elements of the claims is an "illogical and inappropriate process by which to determine the patentability" of the claims of the instant invention over the references identified. *Sensonics Inc. v. Aerosonic Corp.* 38 USPQ2d 1551, 1554 (Fed. Cir. 1996). In view of the foregoing, Applicants respectfully request that the rejections of Claims 1-38 under 35 U.S.C. §103(a) over Kangas *et al.* in view of Redick *et al.* and Connors *et al.* be withdrawn.

As such, Applicants request that the rejection be withdrawn. Applicants request such favorable action on the part of the Examiner.

III. Conclusion

In light of the amendments and comments presented herein, withdrawal of the rejections and reconsideration of the application is respectfully requested. The Applicants respectfully request entry of the foregoing amendments and allowance of all of the pending claims 1-38 in view of the foregoing remarks. The Examiner is invited to telephone the undersigned to discuss any remaining issues so as to expedite the progress of this case toward allowance.

Respectfully submitted,

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